the ability to detect hybridization in the microarray. However, in some instances it may be desirable to ensure that the total area available for hybridization is within a suitable coefficient of variation (CV) from spot to spot. A suitable CV can be less than 1%, less than 5%, less than 10%, less than 15%, less than 20%, or less than 25%. This can be achieved by either making the pitch of the micro-array much smaller than the size of the spot or making sure the width of ridge is much smaller than the pitch or the size of the spot. No precision alignment between the micro-channels on the cover slip and the microarray is necessary.

[0206] **FIG. 44** shows the spot images with two different micro-channel configurations. In **FIG. 44***a*, the channel pitch is similar to the diameter of the probe spot and the channel width is 90% of the pitch. Because the majority of the area on the spot is available for hybridization, the effect of the micro-channel structure on spot to spot uniformity is insignificant. In **FIG. 44***b*, the channel pitch is much smaller than the diameter of the probe spot and the channel width is 50% of the pitch. Although the area available for hybridization is reduced by 50%, the spot to spot signal uniformity is not affected significantly because the channel pitch is much smaller than the spot size. As mentioned before, because the probe molecules are normally in vast oversupply in most applications, the reduction in hybridization area will not significantly affect the ability to detect hybridization in the microarray.

[0207] In a specific example, the diameter of the probe spot on the microarray is 100 μm, the micro-channels have a pitch of 10 μm and a depth and width of 1 μm and 7 μm, respectively. The total volume of liquid needed to fill the micro-channels across the entire cover slip is 0.98 μl. The total sample volume required for hybridization is smaller than 3 μl, even taking liquid pumping into consideration, which is much smaller than that required in most hybridization systems today (~100 μl). The hybridization rate, hence the detection sensitivity can be greatly enhanced due to the increased sample concentration. In addition, because of the very small channel depth which greatly reduces the diffusion distance of target molecules in Z direction, the speed of the hybridization can also be greatly enhanced.

[0208] The micro-channel system described can be used for any liquid to liquid mixing. For example, a different liquid can be loaded into the reservoirs of a microarray and pumped into the micro-channels. By pumping back and forth through the micro-channels, different liquids can be mixed within the micro-channels. The system described can also be used to enhance interactions between target molecules in the liquid and molecules printed on or attached to the surface of the microarray.

[0209] **FIG. 45** illustrates a method of generating movement of target molecules by applying pressure onto a flexible cover slip. In the embodiment shown, the cover slip is formed of an elastic material and one or more movable pins are positioned on top of the flexible cover slip. A tap on the cover slip by one of the pins generates a pressure wave in the liquid sample contained between the cover slip and the substrate slide. The motion of the pins can be programmed in such a way that the sample liquid is pumped to flow in a designed pattern, thus forcing the interaction between the target molecules and the probes. Flow patterns can be switched many times during hybridization to ensure thorough interaction. Pins may move in a vertical or lateral direction in the sample solution, or move in combinations of these two directions.

[0210] Alternatively, the hybridization can be performed without the cover slip, as illustrated in **FIG. 46**. A vibrating pin can be inserted into the liquid sample to improve hybridization directly. Pins can be coated with an inert material such as polytetrafluoroethylene (or Teflon®) to prevent the liquid sample from sticking to the pins. When the cover slip is not used, the hybridization process can be performed in a high humidity chamber to minimize evaporation.

[0211] C. Hybridization Apparatus Having an Inlet for Target Liquid Introduction

[0212] Embodiments of the present invention provide a hybridization apparatus including a hybridization chamber which creates turbulent flow of target liquid while shaking the apparatus so that effective movement of target molecules occurs during hybridization. The hybridization apparatus includes a substrate slide and a cover. The substrate slide has an array of probes deposited on its surface. The cover, as illustrated in one embodiment of this invention in **FIG. 47**, forms a hybridization chamber when it is placed on top of the substrate. The cover may have an adhesive bottom portion that can be firmly adhered on to the substrate slide surface covering the array, as shown in **FIG. 48**. Alternatively, the cover and the substrate slide may be clamped together by two clamps with a gasket on the bottom of the cover as shown in **FIG. 47A**. This hybridization apparatus can he shaken vigorously to generate turbulent flow in the target liquid.

[0213] The hybridization chamber may have, for example, inner chamber dimensions of 20 mm×20 mm×(1.0 mm through 1.75 mm) that take a sample volume of 350-500 μl with a void occupying the rest 100-200 μl equivalent volume in the chamber. This void can help to generate the turbulent flow in the chamber and thus improve hybridization rate and sensitivity.

[0214] In various embodiments, the material of the cover has the following characteristics: first, the material is substantially rigid so that the cover is not deformed in the presence of liquids; second, the material does not absorb target molecules in the sample such as DNA or fluorescent dyes; and third, the material is compatible with the chemicals in the hybridization mix. Materials such as polyethylene may be suitable for the cover. The gasket used may also possess the characteristics listed above. The inner surface of the cover can be coated with a hydrophobic material.

[0215] The cover may be provided with an opening as an inlet on one side or the top of the cover for introducing target liquid. The cover may have another opening as an outlet for removing the target liquid. The inlet and outlet can be closed, for example, by a clamp valve or rubber plugs. One can open the clamp valve or rubber plugs valve to introduce or remove the target liquid. The volume of the target liquid to be introduced should be slightly less than the volume of the chamber volume so that there is a small void in the chamber for allowing the formation of a turbulent flow and an effective movement of target liquid during shaking. The hybridization chamber can be shaken vigorously in a hybridization oven to create good turbulent flow.